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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C09B 67/00, A61K 7/13

A1

(11) International Publication Number: WO 97/19998

(43) International Publication Date: 5 June 1997 (05.06.97)

(21) International Application Number: PCT/DK96/00498

(22) International Filing Date: 29 November 1996 (29.11.96)

(30) Priority Data:
1356/95 30 November 1995 (30.11.95) DK

(71) Applicant (*for all designated States except US*): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): AASLYNG, Dorrit [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). SØRENSEN, Niels, Henrik [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). RØRBÆK, Karen [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: AN ENZYME FOR DYING KERATINOUS FIBRES

(57) Abstract

The present invention relates to a dyeing composition, a method for dying keratinous fibres, in particular hair, fur, hide and wool, and the use of a *Scytalidium* laccase for dyeing.

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Title: An enzyme for dying keratinous fibres

FIELD OF THE INVENTION

The present invention relates to a dyeing composition for keratinous fibres, in particular hair, fur, hide and wool, a method for dying and the use of a *Scytalidium* laccase for dyeing.

BACKGROUND OF THE INVENTION

It has been used for many years to dye the hair to cover appearing grey hair. The need to do so arises from the fact that grey hair is the first sign of having past adolescence, which can be hard to accept for many people.

For instance, in certain parts of Asia it is widely used by both men and women to dye the hair with dyes often referred to by humorous people as "black shoe polish".

During the last few decades hair dyeing has become more and more popular in the western world. At first Punk Rockers and other society critical groups dyed their hair in extreme colours as a part of their protest against the established society, but today especially many young people also uses hair dyes (in more soft tints than the Punk Rockers) as a sort of "cosmetic" to change or freshen up their "look".

Hair dyes

In general hair dyeing compositions on the market today can be divided into three main groups:

- temporary hair dyes,
- semi-permanent hair dyes, and
- permanent oxidative hair dyes.

The temporary hair dyes are only intended to change the natural hair colour for a short period of time and usually functions by depositing dyes on the surface of the hair. Such hair dyes are easy to remove with normal shampooing.

When using semi-permanent hair dyes the colour of the dyed hair can survive for five or more shampooings. This is achieved

by using dyes having a high affinity for hair keratin and which is able to penetrate into the interior of the hair shaft.

Permanent hair dyes are very durable to sunlight, shampooing and other hair treatments and need only to be refreshed once a month as new hair grows out. With these dyeing systems the dyes are created directly in and on the hair. Small aromatic colourless dye precursors (*e.g.* p-phenylene-diamine and o-aminophenol) penetrate deep into the hair where said dye precursors are oxidised by an oxidising agent into coloured polymeric compounds. These coloured compounds are larger than the dye precursors and can not be washed out of the hair.

By including compounds referred to as modifiers (or couplers) in the hair dyeing composition a number of hair colour tints can be obtained. Cathecol and Resorcinol are examples of such modifiers.

Traditionally H_2O_2 is used as the oxidizing agent (colour builder), but also as a bleaching agent. Dyeing compositions comprising H_2O_2 are often referred to as "lightening dyes" due to this lightening effect of H_2O_2.

The use of H_2O_2 in dye compositions have some disadvantages as H_2O_2 damages the hair. Further, oxidative dyeing often demands high pH (normally around pH 9-10), which also inflicts damage on the hair. Consequently, if using dye compositions comprising H_2O_2 it is not recommendable to dye the hair often.

To overcome the disadvantages of using H_2O_2 it has been suggested to use oxidation enzymes to replace H_2O_2.

US patent no. 3,251,742 (Revlon) describes a method for dyeing human hair by dye formation *in situ* (*i.e.* on the hair). An oxidative enzyme is used to the colour formation reactions at a substantially neutral pH (pH 7-8.5).

Laccases, tyrosinases, polyphenolases and catacolases are mentioned as the suitable oxidation enzymes.

EP patent no. 504.005 (Perma S.A.) concerns compositions for dying hair which do not require the presence of H_2O_2 (hydrogen peroxide). The composition comprises an enzyme capable of catalyzing the formation of the polymeric dyes and also dye precursors, such as bases and couplers, in a buffer solution wherein the pH of said composition is between 6.5 and 8 and

said enzyme has an optimal activity in the pH range between 6.5 and 8.

Rhizoctonia praticola laccase and *Rhus vernicifera* laccase have a pH-optimum between 6.5 and 8 and can be used to form the polymeric dyes according to this patent.

Abstract of Papers American Chemical Society vol. 209, no. 1-2, 1995 discloses the cloning of a laccase from a *Scytalidium thermophilum*. The abstract does not mention the use of said laccase for dyeing hair.

SUMMARY OF THE INVENTION

The object of the present invention is to provide improved permanent dyeing compositions for keratinous fibres, in particular hair, fur, hide and wool, which is less damaging to the keratinous fibres than *e.g.* dyeing compositions for hair using H_2O_2.

It has now surprisingly been found that it is possible to provide such an improved dyeing composition by using an enzyme derived from a strain of the filamentous fungus genus *Scytalidium* as the oxidation enzyme.

In the first aspect the invention relates to a permanent dyeing composition for keratinous fibres, in particular hair, fur, hide and wool, comprising an oxidation enzyme comprising
1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,
2) one or more dye precursors, and
optionally 3) one or more modifiers.

In a preferred embodiment of the invention the oxidation enzyme is a laccase derived from a strain of the genus *Scytalidium*, in particular from a strain of the species *Scytalidium thermophilum*.

Secondly, it is the object of the invention to provide a method for dying keratinous fibres, comprising contacting a laccase derived from a strain of the genus Scytalidium with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier for a suitable period of time and under conditions sufficient to permit oxidation of the dye precursor into a coloured compound.

Finally the invention relates to the use of an oxidation enzyme derived from a strain of the genus *Scytalidium* for oxidative dyeing of keratinous fibres, in particular hair, fur, hide and wool.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the dyeing effect of the *Scytalidium thermophilum* laccase (rStL-FXu-1)

DETAILED DESCRIPTION OF THE INVENTION

The object of the present invention is to provide improved permanent dyeing compositions for keratinous fibres, in particular hair, fur, hide and wool, which is less damaging to the keratinous fibres than *e.g.* hair dyeing compositions using H_2O_2.

It has surprisingly be found that it is possible to provide such an improved dyeing composition by using an oxidation enzyme derived from a strain of the filamentous fungus genus *Scytalidium*.

When using said oxidation enzyme derived from a strain of the genus *Scytalidium* the colour developed is as wash stable as oxidative dyeing of *e.g.* hair using H_2O_2 and the light fastness is as good as when dyeing chemically.

Consequently, in the first aspect the present invention relates to a permanent dye composition for keratinous fibres , in particular hair, fur, hide and wool, comprising

1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,

2) one or more dye precursors, and

optionally 3) one or more modifiers.

In an embodiment of the invention the oxidation enzyme is a laccase derived from a strain of genus *Scytalidium*, such as a strain of *Scytalidium thermophilum* *e.g.* the purified laccase described in WO 95/33837 (PCT/US95/06816) from Novo Nordisk, which is hereby incorporated. SEQ ID No 1 shows a DNA sequence encoding a suitable laccase derivable from a strain of the species *Scytalidium thermophilum*.

E. coli JM101 containing the expression vector pShThi5 comprising SEQ ID NO 1 has been deposited under the Budapest

Treaty with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Illinois, 61604. The vector have been given the Accession Number NRRL B-21262.

Also contemplated according to the invention are laccases derived from other microorganisms being more than 80% homologous to SEQ ID NO 1 derived from a strain of the species *Scytalidium thermophilum*.

In addition, *Scytalidium* laccases also encompass alternative forms of laccases which may be found in *S. thermophilum* and as well as laccases which may be found in other fungi which are synonyms of fall within the definition of *S. thermophilum* as defined by Straatsma and Samson, (1993), Mycol. Res. 97, 321-328). These include *S. indonesiacum, Torula thermophila, Humicola brevis* var. thermoidea, *Humicola brevispora, H. grisea* var. thermoidea, *Humicola insolens,* and *Humicola lanuginosa* (also known as *Thermomyces lanuginosus*).

It is to be understood that the *Scytalidium* laccase may be produced homologously, or heterologously using filamentous fungus, yeast or bacteria as the host cell.

Examples of filamentous fungi host cells include strains of the species of *Trichoderma*, preferably a strain of *Trichoderma harzianum* or *Trichoderma reesei*, or a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*, or yeast cells, such as *e.g.* a strain of *Saccharomyces*, in particular *Saccharomyces cerevisiae*, *Saccharomyces kluyveri* or *Saccharomyces uvarum*, a strain of *Schizosaccharomyces* sp., such as *Schizosaccharomyces pombe*, a strain of *Hansenula* sp., *Pichia* sp., *Yarrowia* sp., such as *Yarrowia lipolytica*, or *Kluyveromyces* sp., such as *Kluyveromyces lactis*, or a bacteria, such as gram-positive bacteria such as strains of *Bacillus*, such as strains of *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. megaterium* or *B. thuringiensis*, or strains of *Streptomyces*, such as *S. lividans* or *S. murinus*, or gram-negative bacteria such as *Escherichia coli*.

Laccases (benzenediol:oxygen oxidoreductases) (E.C. class

1.10.3.2 according to Enzyme Nomenclature (1992) Academic Press, Inc) are multi-copper containing enzymes that catalyze the oxidation of phenols. Laccase-mediated oxidations result in the production of aryloxy-radical intermediates from suitable phenolic substrates; the ultimate coupling of the intermediates so produced provides a combination of dimeric, oligomeric, and polymeric reaction products. Certain reaction products can be used to form dyes suitable for dyeing hair (see below).

In an embodiment of the invention the *Scytalidium* laccase is neutral. In the context of laccases of the present invention this means that the pH optimum lies in the range from between 6.0 and 8.0.

To obtain dyeing of the keratinous fibres, such as hair, the dyeing composition of the invention also comprises a dye precursor which is converted into a coloured compound (*i.e.* a dye) by the oxidation agent which according to the invention is an oxidation enzyme derived from a strain of the species *Scytalidium*, such as a strain of *Scytalidium thermophilum*.

Without being limited thereto the dye precursor(s) may be (an) aromatic compound(s) belonging to one of three major chemical families: the diamines, aminophenols (or aminonaphtols) and the phenols. Examples of isatin derivative dye precursors can be found in DE 4,314,317-A1. Further, a number of indole or indoline derivative dye precursors are disclosed in WO 94/00100. Said dye precursors mentioned in these documents are hereby incorporated herein by reference.

Examples of such suitable dye precursors include compounds from the group comprising p-phenylene-diamine (pPD), p-toluylene-diamine, chloro-p-phenylenediamine, p-aminophenol, o-aminophenol and 3,4-diaminotoluene, 2-methyl-1,4-diaminobenzene, 4-methyl-o-phenylenediamine, 2-methoxy-p-phenylenediamine, 2-chloro-1,4-diamino-benzene, 4-amino diphenylamine, 1-amino-4-β-methoxyethylamino-benzene, 1-amino-4-bis-(β-hydroxyethyl)-amonibenzene, 1-3-diamino-benzene, 2-methyl-1,3-diamino-benzene, 2,4-diaminotoluene, 2,6-diaminopyridine, 1-hydroxy-2-amino-benzene, 1-hydroxy-3-amino-benzene, 1-methyl-2-hydroxy-4-amino-benzene, 1-methyl-2-hydroxy-4-β-hydroxyethylamino-benzene, 1-

hydroxy-4-amino-ebnzene, 1-hydroxy-4-methylamino-benzene, 1-methoxy-2,4-diamino-benzene, 1-ethoxy-2,3-diamino-benzene, 1-β-hydroxyethyloxy-2,4-diamino-benzene, phenazines, such as 4,7-phenazinedicarboxylic acid, 2,7-phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diamino-phenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 2,7-diamino 3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol, 2,2'-[(8-amino-2-phenazinyl)imino]bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)- benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]- methanesulfonamide, N-(8-methoxy-2-phenazinyl)- Methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dimethylamino benzoic acid octyl, p-diethoxy amino benzoic amil, p- dipropoxy amino benzoic acid ethyl, acetylsalicylic acid, isatin derivatives, such as 2,3-diamino benzoic acid.

In an embodiment the laccase is used with the dye precursor directly to oxidise it into a coloured compound. The dye precursor may be used alone or in combination with other dye precursors.

However, it is believed that when using a diamine or an aminophenol as the dye precursor at least one of the intermediate in the copolymerisation must be an *ortho-* or *para-*diamine or aminophenol. Examples of such are described in US patent no. 3,251,742 (Revlon), the contents of which are incorporated herein by reference.

Optionally the dyeing composition of the invention (especially hair dyeing compositions) also comprises a modifier (coupler) by which a number of colour tints can be obtained. In general modifiers are used in hair dyeing compositions, as the colours resulting from hair dyeing compositions without modifier(s) are usually unacceptable for most people.

Modifiers are typically m-diamines, m-aminophenols, or polyphenols. The modifier (coupler) reacts with the dye precursor(s) in the presence of the oxidative enzyme, converting it into a coloured compound.

Examples of modifiers (couplers) include m-phenylene-diamine, 2,4-diaminoanisole, 1-hydroxynaphthalene(α-naphthol), 1,4-dihydroxybenzene(hydroquinone), 1,5-dihydroxynapthalene, 1,2-dihydroxybenzene(pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,3-dihydroxy-2-methylbenzene, 1,3-dihydroxy-4-chlorobenzene(4-chlororesorcinol), 1,2,3,trihydroxybenzene, 1,2,4-trihydroxybenzene, 1,2,4-trihydroxy-5-methylbenzene, 1,2,4-trihydroxytoluene.

In the second aspect the invention relates to a method for dying keratinous fibres, in particular hair, fur, hide and wool, comprising contacting a laccase derived from a strain of the genus *Scytalidium* with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier, for a period of time and under conditions sufficient to permit oxidation of the dye precursor into coloured compounds (*i.e.* a dye).

The dyeing method can be conducted with one or more dye precursors, either alone or in combination with one or more modifiers.

The amount of dye precursor(s) and other ingredients used in the composition of the invention are in accordance with usual commercial amounts.

When using a *Scytalidium* laccase, such as the *Scytalidium thermophilum* laccase mentioned above, the method for dyeing keratinous fibres of the invention may be carried out at room temperature, preferably around the optimum temperature of the enzyme, at a pH in the range from 3.0 to 9.0, preferably 4.0 to 8.0, especially pH 6.0 to 8.0.

Suitable dye precursors and optional modifiers are described above.

The use of this *Scytalidium* laccase is an improvement over the more traditional use of H_2O_2 as the latter can damage the keratinous fibres, such as hair. Further, normally prior art methods requires a high pH, which is also damaging to the

keratinous fibres. In contrast hereto, the reaction with laccase can be conducted at acidic or neutral pH, and the oxygen needed for oxidation comes from the air, rather than via harsh chemical oxidation.

The result provided by the use of the *Scytalidium* laccase is comparable to that achieved with use of H_2O_2, not only in colour development, but also in wash stability and light fastness. An additional commercial advantage is that a single container package can be made containing both the laccase and the precursor, in an oxygen free atmosphere, which arrangement is not possible with the use of H_2O_2.

MATERIALS AND METHODS

Materials:

<u>Hair:</u>

6" De Meo Virgin Natural White Hair (De Meo Brothers Inc. US)

Enzymes:

Laccase from *Scytalidium thermophilum* described in WO 95/33837 (PCT/US95/06816) from Novo Nordisk

Deposit of Biological Material

The following biological material has been deposited on the 25th May 1994 under the terms of the Budapest Treaty with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Illinois, 61604 and given the following accession number.

Deposit	Accession Number
E. coli JM101 containing pShTh15	NRRL B-21262.

Dye precursors:

0.1 % w/w p-phenylene-diamine in 0.1 M K-phosphate buffer, pH 7.0. (pPD)

0.1 % w/w p-toluylene-diamine in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w chloro-p-phenylenediamine in 0.1 M K-phosphate

buffer, pH 7.0.
0.1 % w/w p-aminophenol in 0.1 M K-phosphate buffer, pH 7.0.
0.1 % w/w o-aminophenol in 0.1 M K-phosphate buffer, pH 7.0.
0.1 % w/w 3,4-diaminotoluene in 0.1 M K-phosphate, buffer pH 7.0.

Modifiers:
0.1 % w/w m-phenylene-diamine in 0.1 M K-phosphate buffer, pH 7.0.
0.1 % w/w 2,4-diaminoanisole in 0,1 M K-phosphate buffer, pH 7.0.
0.1 % w/w a-naphthol in 0.1 M K-phosphate buffer, pH 7.0.
0.1 % w/w hydroquinone in 0.1 M K-phosphate buffer, pH 7.0.
0.1 % w/w pyrocatechol in 0.1 M K-phosphate buffer, pH 7.0.
0.1% w/w resorcinol in 0.1 M K-phosphate buffer, pH 7.0.
0.1 % w/w 4-chlororesorcinol in 0.1 M K-phosphate buffer, pH 7.0.

The dye precursor is combined with one of the above indicated modifiers so that the final concentration in the dyeing solution is 0.1 % w/w with respect to precursor and 0.1 % w/w with respect to modifier.

Other solutions:
3% H_2O_2 (in the final dye solution)

Commercial shampoo

Equipment:
Minolta CR200 Chroma Meter
Day light bulb: 1000 LUX (D65)

Determination of Laccase Activity (LACU)

Laccase activity is determined from the oxidation of syringaldazin under aerobic conditions. The violet colour produced is photometered at 530 nm. The analytical conditions are 19 mM syringaldazin, 23.2 mM acetate buffer, pH 5.5, 30°C, 1 min. reaction time.

1 laccase unit (LACU) is the amount of enzyme that catalyses

the conversion of 1.0 micromole syringaldazin per minute at these conditions.

Assessment of the hair colour

The quantitative colour of the hair tresses are determined on a Minolta CR200 Chroma Meter by the use the parameters L* ("0"=black and "100"=white), a* ("-"=green and "+"=red) and b* ("-" blue and "+" yellow).

DL*, Da* and Db* are the delta values of L*, a* and b* respectively compared to L*, a* and b* of untreated hair (*e.g.* DL* = $L^*_{sample} - L^*_{untreated\ hair}$).

DE* is calculated as $DE^*=\text{Ö}(DL^{*2}+Da^{*2}+Db^*)$ and is an expression for the total quantitative colour change.

EXAMPLES

Example 1

Dyeing effect

The dyeing effect of a *Scytalidium thermophilum* laccase was tested using the dye precursor o-aminophenol and the modifier m-phenylenediamine.

Hair dyeing

1 gram De Meo white hair tresses were used.

4 ml dye precursor solution (including modifier) is mixed with 1 ml laccase on a Whirley mixer, applied to the hair tresses and incubated at 30°C for 60 minutes.

The hair tresses are then rinsed with running water, washed with shampoo, rinsed with running water, combed, and air dried.

The a*, b* and L* was determined on the Chroma Meter and the DE* values were then calculated.

A hair tress sample treated without enzyme was used as a blind.

The result of the hair dyeing test is shown in figure 1.

Example 2

Wash stability

Tresses of white De Meo hair (1 gram) is used for testing the wash stability of hair dyed using *Scytalidium thermophilum* laccase, compared with hair dyed using H_2O_2, and p-phenylene-diamine (pPD) as the dye precursor. Further the wash stability is compared with a commercial oxidative dye.

The oxidative hair dyeing is carried out as described in Example 1.

Hair wash

The dyed hair tresses are wetted and washed for 15 seconds with 50 ml of commercial shampoo, and rinsed with water for 1 minute and air dried. The hair tresses are washed up to 18 times.

The a*, b* and L* is determined om the Chroma Meter and the ΔE* values are then calculated.

Example 3

The light fastness

Tresses of blond European hair are used for testing the light fastness of hair dyed using *Scytalidium thermophilum* laccase in comparison to hair dyed using H_2O_2. p-phenylene-diamine was used as dye precursor.

The dyeing of the hair was carried out as described in Example 1.

One hair tress is kept dark, while an other is kept at day light (*i.e.* under a day light bulb (D65)), at approximately 1000 LUX) for up to 275 hours.

The a*, b* and L* parameters are determined immediately after the dyeing of the hair, and further during exposure to day light.

DE* then calculated from the determined a*, b* and L* values.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: Bagsvaerd
(D) COUNTRY: Denmark
(E) POSTAL CODE (ZIP): DK-2880
(F) TELEPHONE: +45 4444 8888
(G) TELEFAX: +45 4449 3256

(ii) TITLE OF INVENTION: An enzyme for dying hair

(iii) NUMBER OF SEQUENCES: 2

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2476 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Scytalidium thermophilum

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 349..411

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 502..559

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 632..686

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1739..1804

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join (106..348, 412..501, 560..631, 687..1738, 1805..2194)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Glu Arg Asp Asp His Ser Leu Thr Ser Arg Gln Gly Ser Cys His Ser
40 45 50

CCA AGC AAC CGC GCC TGT TGG TGC TCT GGC TTC GAT ATC AAC ACG GAT 309
Pro Ser Asn Arg Ala Cys Trp Cys Ser Gly Phe Asp Ile Asn Thr Asp
55 60 65

TAT GAG ACC AAG ACT CCA AAC ACC GGA GTG GTG CGG CGG GTTAGTATCC 358
Tyr Glu Thr Lys Thr Pro Asn Thr Gly Val Val Arg Arg
70 75 80

CAAGTTACGT TTGACCAAGA AATGGACGTG AAGTGTGCTG ACTCTCCCGC TAG 411

TAC ACC TTT GAT ATC ACC GAA GTC GAC AAC CGC CCC GGT CCC GAT GGG 459
Tyr Thr Phe Asp Ile Thr Glu Val Asp Asn Arg Pro Gly Pro Asp Gly
85 90 95

GTC ATC AAG GAG AAG CTC ATG CTT ATC AAC GAC AAA CTC CTG GTAGG 506
Val Ile Lys Glu Lys Leu Met Leu Ile Asn Asp Lys Leu Leu
100 105 110

GTCCTCTCGA ACGCCTGCGT CTGCCACACA GCGTAAAACT AACGAACCGC TAG 559

GGC CCG ACA GTC TTC GCA AAC TGG GGC GAC ACC ATC GAG GTG ACC GTC 607
Gly Pro Thr Val Phe Ala Asn Trp Gly Asp Thr Ile Glu Val Thr Val
115 120 125

AAC AAC CAC CTG AGA ACC AAC GGA GTAAGCGTTC GGACACAAAG CCCAGCAACC 661
Asn Asn His Leu Arg Thr Asn Gly
130 135

TAGACACACT CAACTGACCA AGTAG ACC TCC ATC CAC TGG CAC GGC TTG CAC CAA 716
Thr Ser Ile His Trp His Gly Leu His Gln
140 145

AAA GGA ACC AAC TAC CAC GAC GGC GCC AAC GGC GTG ACC GAG TGT CCC 764
Lys Gly Thr Asn Tyr His Asp Gly Ala Asn Gly Val Thr Glu Cys Pro
150 155 160

ATC CCG CCC GGT GGC TCC CGA GTC TAC AGC TTC CGA GCG CGC CAA TAT 812
Ile Pro Pro Gly Gly Ser Arg Val Tyr Ser Phe Arg Ala Arg Gln Tyr
165 170 175

GGA ACG TCA TGG TAC CAC TCC CAC TTC TCC GCC CAG TAT GGC AAC GGC 860
Gly Thr Ser Trp Tyr His Ser His Phe Ser Ala Gln Tyr Gly Asn Gly
180 185 190

GTG AGC GGC GCC ATC CAG ATC AAC GGA CCC GCC TCC CTG CCC TAC GAC 908
Val Ser Gly Ala Ile Gln Ile Asn Gly Pro Ala Ser Leu Pro Tyr Asp
195 200 205

ATC GAC CTC GGC GTC CTC CCG CTG CAG GAC TGG TAC TAC AAG TCC GCC 956
Ile Asp Leu Gly Val Leu Pro Leu Xaa Asp Trp Tyr Tyr Lys Ser Ala
210 215 220 225

GAC CAG CTC GTC ATC GAG ACC CTG GCC AAG GGC AAC GCT CCG TTC AGC 1004
Asp Gln Leu Val Ile Glu Thr Leu Xaa Lys Gly Asn Ala Pro Phe Ser
230 235 240

GAC AAC GTC CTC ATC AAC GGC ACC GCA AAG CAC CCC ACC ACT GGC GAA 1052
Asp Asn Val Leu Il Asn Gly Thr Ala Lys His Pro Thr Thr Gly Glu
245 250 255

GGG GAG TAC GCC ATC GTG AAG CTC ACC CCG GGC AAA CGC CAT CGC CTG 1100
Gly Glu Tyr Ala Ile Val Lys Leu Thr Pro Asp Lys Arg His Arg Leu

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        260                 265                 270

CGG CTC ATC AAC ATG TCG GTG GAG AAC CAC TTC CAG GTC TCG CTG GCG      1148
Arg Leu Ile Asn Met Ser Val Glu Asn His Phe Gln Val Ser Leu Ala
    275                 280                 285

AAG CAC ACC ATG ACG GTC ATC GCG GCG GAC ATG GTC CCC GTC AAC GCC      1196
Lys His Thr Met Thr Val Ile Ala Ala Asp Met Val Pro Val Asn Ala
290                 295                 300                 305

ATG ACC GTC GAC AGC CTG TTT ATG GCC GNC GGG CAG CGG TAT GAT GTT      1244
Met Thr Val Asp Ser Leu Phe Met Ala Val Gly Gln Arg Tyr Asp Val
            310                 315                 320

ACC ATC GAC GCG AGC CAG GCG GTG GGG AAT TAC TGG TTC AAC ATC ACC      1292
Thr Ile Asp Ala Ser Gln Ala Val Gly Asn Tyr Trp Phe Asn Ile Thr
        325                 330                 335

TTT GGA GGG CAG CAG AAG TGC GGC TTC TCG CAC AAT CCG GCG CCG GCA      1340
Phe Gly Gly Gln Gln Lys Cys Gly Phe Ser His Asn Pro Ala Pro Ala
        340                 345                 350

GCC ATC TTT CGC TAC GAG GGC GCT CCT GAC GCT CTG CCG ACG GAT CCT      1388
Ala Ile Phe Arg Tyr Glu Gly Ala Pro Asp Ala Leu Pro Thr Asp Pro
    355                 360                 365

GGC GCT GCG CCA AAG GAT CAT CAG TGC CTG GAC ACT TTG GAT CTT TCA      1436
Gly Ala Ala Pro Lys Asp His Gln Cys Leu Asp Thr Leu Asp Leu Ser
370                 375                 380                 385

CCG GTG GTG CAA AAG AAC GTG CCG GTT GAC GGG TTC GTC AAA GAG CCT      1484
Pro Val Val Gln Lys Asn Val Pro Val Asp Gly Phe Val Lys Glu Pro
            390                 395                 400

GGC AAT ACG CTG CCG GTG ACG CTC CAT GTT GAC CAG GCC GCG GCT CCA      1532
Gly Asn Thr Leu Pro Val Thr Leu His Val Asp Gln Ala Ala Ala Pro
        405                 410                 415

CAC GTG TTT ACG TGG AAG ATC AAC GGG AGC GCT GCG GAC GTG GAC TGG      1580
His Val Phe Thr Trp Lys Ile Asn Gly Ser Ala Ala Asp Val Asp Trp
        420                 425                 430

GAC AGG CCG GTG CTG GAG TAT GTC ATG AAC AAT GAC CTG TCT AGC ATT      1628
Asp Arg Pro Val Leu Glu Tyr Val Met Asn Asn Asp Leu Ser Ser Ile
    435                 440                 445

CCG GTC AAG AAC AAC ATT GTG AGG GTG GAC GGA GTC AAC GAG TGG ACG      1676
Pro Val Lys Asn Asn Ile Val Arg Val Asp Gly Val Asn Glu Trp Thr
450                 455                 460                 465

TAC TGG CTC GTC GAA AAC GAC CCG GAG GGC CGC CTC AGT TTG CCG CAT      1724
Tyr Trp Leu Val Glu Asn Asp Pro Glu Gly Arg Leu Ser Leu Pro His
            470                 475                 470

CCG ATG CAT CTA CAC GTAAGTCACA TCCCCCACTA CCATTCGGAA TGACCACCAG      1779
Pro Met His Leu His
            475

GTACTGACAC CCTCCTCCTC AATAG GGA CAC GAT TTC TTT GTC CTA GGC CGC      1831
                            Gly His Asp Phe Phe Val Leu Gly Arg
                                        480                 485

TCC CCC GAC GTC TCG CCC GAT TCA GAA ACC CGC TTC GTC TTT GAC CCG      1879
Ser Pro Asp Val Ser Pro Asp Ser Glu Thr Arg Phe Val Phe Asp Pro
            490                 495                 500

GCC GTC GAC CTC CCC CGT CTG CGC GGA CAC AAC CCC GTC CGG CGC GAC      1927
Ala Val Asp Leu Pro Arg Leu Arg Gly His Asn Pro Val Arg Arg Asp
            505                 510                 515
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GTC ACC ATG CTT CCC GCG CGC GGC TGG CTG CTG CTG GCC TTC CGC ACG      1975
Val Thr Met Leu Pro Ala Arg Glu Trp Leu Leu Leu Ala Phe Arg Thr
        520                 525                 530

GAC AAC CCG GGC GCG TGG TTG TTC CAC TGC CAC ATC GCG TGR CAC GTG      2023
Asp Asn Pro Gly Ala Trp Leu Phe His Cys His Ile Ala Trp His Val
    535                 540                 545

TCG GGC GGG TTA AGC GTC GAC TTT CTG GAG CGG CCG GAC GAG CTG CGC      2071
Ser Gly Gly Leu Ser Val Asp Phe Leu Glu Arg Pro Asp Glu Leu Arg
550                 555                 560                 565

GGG CAG CTG ACG GGA GAG AGC AAG GCG GAG TTG GAG CGT GTT TGT CGC      2119
Gly Gln Leu Thr Gly Glu Ser Lys Ala Glu Leu Glu Arg Val Cys Arg
                570                 575                 580

GAG TGG AAG GAT TGG GAG GCG AAG AGC CCG CAT GGG AAG ATC GAT TCG      2167
Glu Trp Lys Asp Trp Glu Ala Lys Ser Pro His Gly Lys Ile Asp Ser
            585                 590                 595

GGG TTG AAG CAG CGG CGA TGG GAT GCG TGAGGTAGTT GGGCGGATTG            2214
Gly Leu Lys Gln Arg Arg Trp Asp Ala
        600                 605

TTTAACACGT AGTGGGTAAG GTTGGGGCGG GTTTGTTTGG CGTTTTCAGG GGTTGGGGTG    2274

CGGATGCTGG TCATCCGGGA AACGGCTCTA CAACTGGTGT CAATAGACTA ATATAGAGTG    2334

ATCAAAGAAC TGAGGTTCTG AAAGAGGCGT GGAAGTCGCG TTGTGACTCC CTTTGCCATG    2394

TTGGGAAGTG TGGCTCAACA TTGTGTTCAG GTTTGCTCAG GGTGATNTCG AACTGACGTN    2454

TTGATGAGGG TTATTGCNTA GA                                             2476
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 616 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Scytalidium thermophilum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Lys Arg Phe Phe Ile Asn Ser Leu Leu Leu Leu Ala Gly Leu Leu
1               5                   10                  15

Asn Ser Gly Ala Leu Ala Ala Pro Ser Thr His Pro Arg Ser Asn Pro
            20                  25                  30

Asp Ile Leu Leu Glu Arg Asp Asp His Ser Leu Thr Ser Arg Gln Gly
        35                  40                  45

Ser Cys His Ser Pro Ser Asn Arg Ala Cys Trp Cys Ser Gly Phe Asp
    50                  55                  60

Ile Asn Thr Asp Tyr Glu Thr Lys Thr Pro Asn Thr Gly Val Val Arg
65                  70                  75                  80

Arg Tyr Thr Phe Asp Ile Thr Glu Val Asp Asn Arg Pro Gly Pro Asp
                85                  90                  95
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Gly Val Ile Lys Glu Lys Leu Met Leu Ile Asn Asp Lys Leu Leu Gly
            100                 105                 110

Pro Thr Val Phe Ala Asn Trp Gly Asp Thr Ile Glu Val Thr Val Asn
        115                 120                 125

Asn His Leu Arg Thr Asn Gly Thr Ser Ile His Trp His Gly Leu His
    130                 135                 140

Gln Lys Gly Thr Asn Tyr His Asp Gly Ala Asn Gly Val Thr Glu Cys
145                 150                 155                 160

Pro Ile Pro Pro Gly Gly Ser Arg Val Tyr Ser Phe Arg Ala Arg Gln
                165                 170                 175

Tyr Gly Thr Ser Trp Tyr His Ser His Phe Ser Ala Gln Tyr Gly Asn
            180                 185                 190

Gly Val Ser Gly Ala Ile Gln Ile Asn Gly Pro Ala Ser Leu Pro Tyr
        195                 200                 205

Asp Ile Asp Leu Gly Val Leu Pro Leu Gln Asp Trp Tyr Tyr Lys Ser
    210                 215                 220

Ala Asp Gln Leu Val Ile Glu Thr Leu Ala Lys Gly Asn Ala Pro Phe
225                 230                 235                 240

Ser Asp Asn Val Leu Ile Asn Gly Thr Ala Lys His Pro Thr Thr Gly
                245                 250                 255

Glu Gly Glu Tyr Ala Ile Val Lys Leu Thr Pro Asp Lys Arg His Arg
            260                 265                 270

Leu Arg Leu Ile Asn Met Ser Val Glu Asn His Phe Gln Val Ser Leu
        275                 280                 285

Ala Lys His Thr Met Thr Val Ile Ala Ala Asp Met Val Pro Val Asn
    290                 295                 300

Ala Met Thr Val Asp Ser Leu Phe Met Ala Xaa Gly Gln Arg Tyr Asp
305                 310                 315                 320

Val Thr Ile Asp Ala Ser Gln Ala Val Gly Asn Tyr Trp Phe Asn Ile
                325                 330                 335

Thr Phe Gly Gly Gln Gln Lys Cys Gly Phe Ser His Asn Pro Ala Pro
            340                 345                 350

Ala Ala Ile Phe Arg Tyr Glu Gly Ala Pro Asp Ala Leu Pro Thr Asp
        355                 360                 365

Pro Gly Ala Ala Pro Lys Asp His Gln Cys Leu Asp Thr Leu Asp Leu
    370                 375                 380

Ser Pro Val Val Gln Lys Asn Val Pro Val Asp Gly Phe Val Lys Glu
385                 390                 395                 400

Pro Gly Asn Thr Leu Pro Val Thr Leu His Val Asp Gln Ala Ala Ala
                405                 410                 415

Pro His Val Phe Thr Trp Lys Ile Asn Gly Ser Ala Ala Asp Val Asp
            420                 425                 430

Trp Asp Arg Pro Val Leu Glu Tyr Val Met Asn Asn Asp Leu Ser Ser
        435                 440                 445

Ile Pro Val Lys Asn Asn Ile Val Arg Val Asp Gly Val Asn Glu Trp
    450                 455                 460
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Thr Tyr Trp Leu Val Glu Asn Asp Pro Glu Gly Arg Leu Ser Leu Pro
465                 470                 475                 480

His Pro Met His Leu His Gly His Asp Phe Phe Val Leu Gly Arg Ser
                485                 490                 495

Pro Asp Val Ser Pro Asp Ser Glu Thr Arg Phe Val Phe Asp Pro Ala
            500                 505                 510

Val Asp Leu Pro Arg Leu Arg Gly His Asn Pro Val Arg Arg Asp Val
        515                 520                 525

Thr Met Leu Pro Ala Arg Gly Trp Leu Leu Leu Ala Phe Arg Thr Asp
    530                 535                 540

Asn Pro Gly Ala Trp Leu Phe His Cys His Ile Ala Trp His Val Ser
545                 550                 555                 560

Gly Gly Leu Ser Val Asp Phe Leu Glu Arg Pro Asp Glu Leu Arg Gly
                565                 570                 575

Gln Leu Thr Gly Glu Ser Lys Ala Glu Leu Glu Arg Val Cys Arg Glu
            580                 585                 590

Trp Lys Asp Trp Glu Ala Lys Ser Pro His Gly Lys Ile Asp Ser Gly
        595                 600                 605

Leu Lys Gln Arg Arg Trp Asp Ala
    610                 615
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 *bis*)

A. The indications made below relate to the microorganism referred to in the description on page 9, line 21-31.

B. IDENTIFICATION OF — Further deposits are identified on an additional sheet ☐

Name of depository institution

Agricultural Research Service Patent Culture Collection (NRRL)

Address of depository institution (*including postal code and country*)

Northern Regional Research Center
1815 University Street
Peoria, IL 61604, US

Date of deposit	Accession Number
25 May 1994	NRRL B-21262

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) — This information is continued on an additional sheet ☐

In respect of those designations in which a European and/or Australia Patent is sought, during the pendency of the patent application, a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (Rule 28(4) EPC/Regulation 3.25 of Australia Statutory Rule 1991 No. 71).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indication listed below will be submitted to the International Bureau Later (*specify the general nature of the indications e.g. "Accession Number of Deposit"*)

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received with the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1992)

PATENT CLAIMS

1. A dyeing composition comprising an oxidation enzyme characterised in that the composition comprises:
1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,
2) one or more dye precursors, and
optionally 3) one or more modifiers.

2. The dyeing composition according to claim 1, wherein the oxidation enzyme is derived from a strain of the genus *Scytalidium* laccase

3. The dyeing composition according to claim 2, wherein the laccase is derived from a strain of the species *Scytalidium thermophilum*.

4. The dyeing composition according to claims 2 and 3, wherein the laccase is neutral.

5. The dyeing composition according to claim 3, having the sequence shown in SEQ ID No 1.

6. The dyeing composition according to claim 5, wherein the sequence encoding the laccase is homologous to the SEQ ID NO 1.

7. The dyeing composition according to claim 6, wherein the sequence encoding the laccase is more than 80% homologous to SEQ ID NO 1.

8. The dyeing composition according to any of claims 1 to 7, comprising a dye precursor selected from the group comprising p-phenylene-diamine (pPD), p-toluylene-diamine, chloro-p-phenylenediamine, p-aminophenol, o-aminophenol and 3,4-diaminotoluene, 2-methyl-1,4-diaminobenzene, 4-methyl-o-phenylenediamine, 2-methoxy-p-phenylenediamine, 2-chloro-1,4-diamino-benzene, 4-amino diphenylamine, 1-amino-4-β-methoxyethylamino-benzene, 1-amino-4-bis-(β-hydroxyethyl)-amonibenzene, 1-3-diamino-benzene, 2-methyl-1,3-diamino-benzene, 2,4-diaminotoluene, 2,6-diaminopyridine, 1-hydroxy-2-amino-benzene, 1-hydroxy-3-amino-benzene, 1-methyl-2-hydroxy-4-amino-benzene, 1-methyl-2-hydroxy-4-β-hydroxyethylamino-benzene, 1-hydroxy-4-amino-benzene, 1-hydroxy-4-methylamino-benzene, 1-methoxy-2,4-diamino-benzene, 1-ethoxy-2,3-diamino-benzene, 1-β-hydroxyethyloxy-2,4-diamino-

benzene, phenazines, such as 4,7-phenazinedicarboxylic acid, 2,7-phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diaminophenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 2,7-diamino 3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol, 2,2'-[(8-amino-2-phenazinyl)imino]bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)- benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]- methanesulfonamide, N-(8-methoxy-2-phenazinyl)- Methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dimethylamino benzoic acid octyl, p-diethoxy amino benzoic amil, p- dipropoxy amino benzoic acid ethyl, acetylsalicylic acid, isatin derivatives, such as 2,3-diamino benzoic acid.

9. The dyeing composition according to claims 8, comprising a dye modifier selected from the group comprising m-phenylene-diamine, 2,4-diaminoanisole, 1-hydroxynaphthalene(α-naphthol), 1,4-dihydroxybenzene(hydroquinone), 1,5-dihydroxynapthalene, 1,2-dihydroxybenzene(pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,3-dihydroxy-2-methylbenzene, 1,3-dihydroxy-4-chlorobenzene (4-chlororesorcinol), 1,2,3,trihydroxybenzene, 1,2,4-trihydroxybenzene, 1,2,4-trihydroxy-5-methylbenzene, 1,2,4-trihydroxytoluene.

10. A method for dying comprising contacting a laccase derived from a strain of the genus *Scytalidium* with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier for a period of time and under conditions sufficient to permit oxidation of the dye precursor into a coloured compound.

11. The method according to claim 10, wherein the dyeing is carried out at a pH in the range from 3.0 to 9.0, preferably 4.0 to 8.0, especially 6.0 to 8.0.

12. Use of an oxidation enzyme derived from a strain of the genus *Scytalidium* for oxidative dyeing keratinous fibres, in particular hair, fur, hide and wool.

13. The use according to claim 14, wherein the oxidation enzyme is derived from a strain of the species *Scytalidium thermophilum*.

1/1

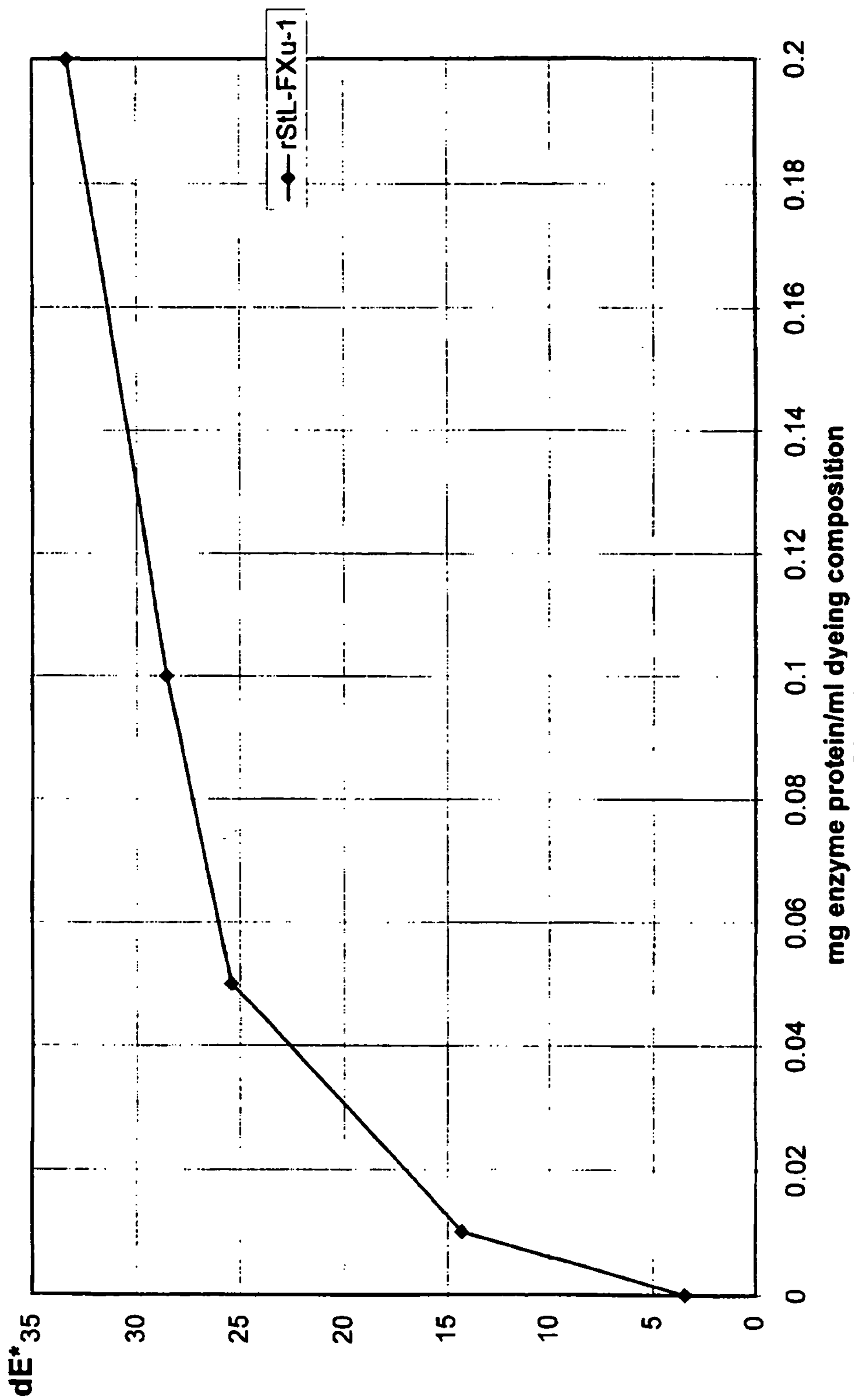
dE*
35
30
25
20
15
10
5
0
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
mg enzyme protein/ml dyeing composition
rStL-FXu-1

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00498

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C09B 67/00, A61K 7/13
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C09B, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9533837 A1 (NOVO NORDISK BIOTECH, INC.), 14 December 1995 (14.12.95), claims 28, 29; page 15, line 34 - page 16	1-13
	--	
P,A	WO 9533836 A1 (NOVO NORDISK BIOTECH, INC.), 14 December 1995 (14.12.95), claims 31-42; page 16, line 12 - page 17, line 27; page 34, line 20 - page 36	1-13
	--	
X	EP 0504005 A1 (PERMA SOCIETE ANONYME), 16 Sept 1992 (16.09.92)	1-13
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 1997	01 -03- 1997
Name and mailing address of the ISA/ Swedish Patent Offic Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Strandell Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00498

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3251742 A (SAUL SOLOWAY), 17 May 1966 (17.05.66) --	1-13
X	WO 9600290 A1 (NOVO NORDISK BIOTECH, INC.), 4 January 1996 (04.01.96), claims 37-48; page 48, line 25 - page 54, line 24 --	1-13
X	STN International, File CAPLUS, CAPLUS accession no. 1991:498981, Saruno, Rinjiro: "Hair- dyeing preparations containing melanin or other polyphenol pigments and manufacture of the pigments"; & JP,A2,910403 --	1-13
X	STN International, File CAPLUS, CAPLUS accession no. 1995:974547, Chivukula, Muralikrishna et al: "Phenolic azo dye oxidation by laccase from Pyricularia oryzae"; & Appl. Environ. Microbiol. (1995), 61(12), 4374-77 --	1-13
A	DE 4314317 A1 (HENKEL KGAA), 3 November 1994 (03.11.94) --	8
A	WO 9400100 A1 (L'OREAL), 6 January 1994 (06.01.94) --	8
A	WO 9507988 A1 (NOVO NORDISK A/S), 23 March 1995 (23.03.95), claim 41 -- --------	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/02/97

International application No.
PCT/DK 96/00498

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9533837	14/12/95	AU-A- 2656695	04/01/96
WO-A1- 9533836	14/12/95	AU-A- 2656595	04/01/96
EP-A1- 0504005	16/09/92	AT-T- 121931	15/05/95
		CA-A- 2061826	09/09/92
		DE-D,T- 69202290	09/11/95
		ES-T- 2072720	16/07/95
		FR-A,B- 2673534	11/09/92
		JP-A- 6172145	21/06/94
US-A- 3251742	17/05/66	FR-A- 1363462	00/00/00
		GB-A- 993923	00/00/00
WO-A1- 9600290	04/01/96	AU-A- 2827895	19/01/96
DE-A1- 4314317	03/11/94	EP-A- 0695162	07/02/96
		JP-T- 8509478	08/10/96
		WO-A- 9424988	10/11/94
WO-A1- 9400100	06/01/94	DE-D,T- 69301464	05/06/96
		EP-A,B- 0645999	05/04/95
		FR-A,B- 2692782	31/12/93
		JP-T- 7508271	14/09/95
		US-A- 5538517	23/07/96
WO-A1- 9507988	23/03/95	AU-A- 7833694	03/04/95
		CA-A- 2171288	23/03/95
		CN-A- 1133067	09/10/96
		EP-A- 0719337	03/07/96
		FI-A- 961250	18/03/96
		US-A- 5480801	02/01/96